**Claims**

1. An analytical device for performing immunoassay for the detection of analyte in a liquid sample comprising:
   (a) a reaction membrane which is liquid-permeable and porous and having an upper and lower surface, an exposed area of the said upper surface having immobilized therein an antibody or antigen capable of binding to the target analyte said immobilized antibody or antigen being concentrated in a multiple spotted region of said upper surface,
   (b) a semi-rigid liquid-impervious bottom support layer wherein a portion of lower surface of the said reaction membrane in breadth corner having no immobilized antibody or antigen, is attached to upper surface of support layer by water-insoluble adhesive or tape having glue on both sides, and
   (c) a body of absorbent material having an upper surface and lower surface, capable of absorbing liquid are provided separately.

2. The analytical device as claimed in claim 1 is alternately comprising a narrow solid strip of a liquid-impervious body placed in-between reaction membrane and semi-rigid support layer wherein upper surface is attached to a portion of lower surface of the said reaction membrane in breadth corner and lower surface to bottom support layer by water-insoluble adhesive.

3. The analytical device as claimed in claim 1 wherein, the size and periphery of the said reaction membrane is much smaller than the said bottom support layer.

4. The analytical device as claimed in claim 1 wherein, said upper surface of said absorbent body provided separately extends beyond the periphery of said reaction membrane but smaller than the said bottom support layer.

5. The analytical device as claimed in claim 1 wherein, said absorbent body is not fitted together with reaction membrane using compression or adhesives during manufacture.

6. The analytical device as claimed in claim 2 wherein, the said narrow solid-strip thickness is similar or higher than absorbent body.

7. The analytical device as claimed in claim 1 wherein, the size of the reaction membrane is not critical and bigger size can be used in a single device to perform batch of samples.

8. The analytical device as claimed in claim 2 wherein multiple strips of the reaction membrane can be attached to semi-rigid support layer to perform immunoassay on batch of samples.

9. The analytical device as claimed in claim 1 wherein, the said reaction membrane is selected from nitrocellulose, other variety of semi-permeable membrane materials including nylon, polyvinyledine difloride and other similar materials.

10. The analytical device as claimed in claim 1 wherein, the average diameter of the reaction membrane is in the range of about 0.22 to about 3 microns and preferably 0.45 microns.

11. The analytical device as claimed in claim 1 wherein, the loose area of the reaction membrane has immobilized thereon the antibody or antigen over entire membrane surface as multiple dots or under certain circumstances, it is desirable to immobilize across the entire membrane surface at uniform concentration.

12. The analytical device as claimed in claim 1 wherein, it is capable of immobilizing more than one specific antibody to the membrane in the same or different areas for simultaneous detection of multiple analyte in a sample with a single assay device.

13. The analytical device as claimed in claim 1 wherein, the unused binding sites on nitrocellulose are blocked with suitable blocking proteins selected from casein, BSA, gelatin and other similar materials.

14. The analytical device as claimed in claim 1 wherein, for ultrasensitive format vacant binding sites on nitrocellulose membrane are blocked with electron rich blocking proteins such as p-hydoxy-phenylpropionic acid-casein conjugate, p-hydroxy-phenylpropionic acid-gelatin conjugate and other similar materials for application of Super-CARD signal amplification.

15. The analytical device as claimed in claim 1 wherein, the bottom support layer with adequate mechanical strength is selected from the group consisting of polyethylene, plastic and fiberglass.

16. The analytical device as claimed in claim 1 wherein, the reaction membrane is attached over bottom support layer using water insoluble adhesive applied in the top 4mm lower portion of the membrane.

17. The analytical device as claimed in claim 1 wherein, adhesive tape having glue on both sides may also be used to attach membrane over bottom support layer.

18. The analytical device as claimed in claim 1 wherein, the separately provided absorbent body is selected from the group consisting of cellulose acetate, filter paper, bathroom tissue paper and other similar absorbent materials.

19. The analytical device as claimed in claim 1 wherein, the thickness of the said absorbent body is ranging from about 0.1 to 8.0 mm and more.

20. The analytical device as claimed in claim 1 wherein, a single analytical device contains more than one disposable absorbent body.

21. The analytical device as claimed in claim 1 wherein, absorbent body is changed for addition of elements of signal amplification reagents.

22. The analytical device as claimed in claim 1 wherein, assembling of absorbent body for performing immunoassay comprising:
    (a) soaking the absorbent body with liquid and assembled in such a way that upper surface of the absorbent body is in intimate contact with lower surface of the reaction membrane and upper surface over bottom support layer,
    (b) removing the entrapped air in between lower and upper surface of reaction membrane and absorbent body by pressing the upper surface of the reaction membrane,
    (c) the void volume of reaction membrane is saturated and the distance separating the reactive membrane and the absorbent body is such that networks of capillary channels is formed were the two members are in contact,
    (d) the flow of applied sample or reagent is always downwards and focused without application of any force to the absorbent body , and

(e) the void volume of the wetted absorbent body is still sufficient to substantially fill the additional volume of fluid introduced during assay.

23. The assay method as claimed in claim 22 wherein, absorbent body is soaked in deionized distilled water, buffer and other similar materials.

24. The assay method as claimed in claim 22 wherein, the upper surface of the reaction membrane is pressed with small roller, rim-less small test-tube and other similar tools.

25. The assay method as claimed in claim 22 wherein, pre-wetted absorbent body saturates the void volume of reaction membrane, which thereby does not allow spread of sample or immunoassay reagents.

26. The assay method as claimed in claim 22 wherein, the costly-labeled reagents are used efficiently.

27. The assay method as claimed in claim 22 wherein, 10 to 100μl of sample or labeled reagents are used.

28. The assay method as claimed in claim 22 wherein, multiple of 10 to 100μl of sample or labeled reagents are applied.

29. The assay method as claimed in claim 22 wherein, the label reagent is premixed with standard or sample prior to addition to different areas of the membrane in the device or it can be added after standard or sample addition.

30. The assay method as claimed in claim 22 wherein, more than one specific antibody is immobilized in the same or different areas for simultaneous detection of multiple analytes in a sample with a single assay device.

31. The assay method as claimed in claim 22 wherein, after application of samples and reagents absorbent body is discarded.

32. The assay method as claimed in claim 22 wherein, the reaction membrane is washed directly over device with the help of wash bottle.

33. The assay method as claimed in claim 22 wherein, for high sensitivity elements of signal amplification are further added.

34. The assay method as claimed in claim 22 wherein, the signal amplification method like Super-CARD is applied.

35. The assay method as claimed in claim 22 wherein, biotinylated tyramine is added directly over reaction membrane in the device.

36. The assay method as claimed in claim 22 wherein, a fresh pre-wetted absorbent body is assembled and avidin-peroxidase conjugate is added.

37. The assay method as claimed in claim 36 wherein, the substrate solution added directly over reaction membrane produced color spots within well-defined area.

38. The assay method as claimed in claim 37 wherein, the exposed area of the reaction membrane is sufficiently greater to allow visualization of the intensity of the color spots.

39. The assay method as claimed in claim 38 wherein, visual comparison with known concentration in reference standard gives semi-quantitative estimate of the amount of antigen present in the sample.

40. The assay method as claimed in claim 36 wherein, the substrate solution is added directly without application of signal amplification.

41. The assay method as claimed in claim 36 wherein, signal amplification step is not necessary for that analyte which is present in high concentration.

42. The assay method as claimed in claims 22 and 36 wherein, the assay results is obtained within 3 to 10 minutes.

43. The assay method as claimed in claim 22 wherein, the analyte is selected from the group consisting of antigens, antibodies, haptens, drugs, hormones, macromolecules, toxins, bacteria, viruses, enzymes, tumor markers, environmental pollutants, nucleic acids and other natural receptors.